Aquaculture 287 (2009) 180–184

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds

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ARTICLE INFO

Article history:
Received 8 September 2008
Received in revised form 16 October 2008
Accepted 17 October 2008

Keywords:
Rainbow trout
Nutrition
Amino acid
Protein
Plant-based feeds

ABSTRACT

Reducing dietary protein in trout feeds will reduce production costs if growth performance can be maintained. A study was conducted to determine if balancing plant-based diets on an available amino acid basis to the profile of trout muscle would result in equal performance with a reduction in total protein level. The diets were formulated to contain either 45 or 35% intact crude protein (45CP and 35CP diets, respectively). To these basal diets, lysine, methionine, threonine and glycine were supplemented to be equivalent to 450 g/kg protein from rainbow trout muscle (45AA and 35AA, respectively) on an available amino acid basis. A fifth diet was formulated to contain 35% crude protein from plant proteins and supplemented as for the 35AA diet but only with lysine, methionine and threonine (35AA-Gly). The diets were fed to apparent satiation to triplicate tanks of 25, 20 g rainbow trout for a period of 12-weeks. Amino acid supplementation improved weight gain by 11% in the 45AA diet over the 45CP diet and by 11.6 and 15% in the 35AA and 35AA-Gly diets relative to the 35CP diet. Feed conversion ratio was poorest for the 35CP diet compared to other treatments. Protein retention efficiencies were improved in the 35AA and 35AA-Gly dietary treatments compared to the 45CP treatment. Intraperitoneal fat ratio decreased with amino acid supplementation at both crude protein levels. Muscle ratio increased by 10% when amino acids were supplemented to the 45CP diet and by an average of 13.6% when amino acids were supplemented to the 35CP diet. Amino acid supplementation reduced the retention efficiency of lysine with no affects on methionine and threonine. Retention efficiencies of isoleucine and leucine were increased by amino acid supplementation at both basal protein levels. Glycine supplementation had no beneficial effects on fish performance. In conclusion, dietary crude protein content of plant-based diets for rainbow trout can be reduced from 46 to 41.5% by supplementing lysine, methionine, and threonine with no reduction in growth and an improvement in protein retention efficiency and muscle ratio.

Published by Elsevier B.V.

1. Introduction

Reducing the protein content of fish feeds is one strategy to increase the sustainability of trout aquaculture via reducing feed costs as well as reducing the environmental impact if economical growth can be maintained with less nitrogen input. Animal growth is determined by the first limiting factor be it environmental, nutritional, or genetics. The physiological state of the animal also keeps nutrient requirements in constant flux as requirements may change with environmental conditions, perturbations to the health status, and the size or age of the animal (Lovell, 2002; Wilson, 2002).

Protein requirements are the sum of amino acid demands for protein deposition, biosynthesis of metabolic intermediates, and catabolism of substrates for energy. Requirements for the 10 essential amino acids have been established for rainbow trout (Hardy, 2002) and plant-based diets generally do not meet these requirements. Multiple research groups have noted that supplementation of amino acids will improve performance of trout (Davies and Morris, 1997; Yamamoto et al., 2005). However, the exact balance of amino acids that allows peak growth and production efficiencies has not been determined. One method used to estimate requirements of amino acids and define balance has been the comparison of dietary amino acid ratios with those of tissues from the species being cultured. This so called “Ideal Protein” has been widely studied and used in the production animal industries (Fuller et al., 1989; Wang and Fuller, 1989; Boisen et al., 2000; Dari et al., 2005). In brief, this theory is based on the hypothesis that amino acids for growth and metabolism must be provided to the animal in a defined ratio to achieve peak performance and amino acid retention, though some debate exists as to which tissues from the animal of interest most appropriately

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[1] This study was funded by the USDA/Agricultural Research Service Project No. 5366-21310-003-00D.

0044-8486/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aquaculture.2008.10.037

Table 1
Diet formulations and proximate composition of the experimental diets[a]

Ingredient	45CP	45AA	35CP	35AA	35AA-Gly
	g/kg dry weight				
Corn gluten meal	140	140	108.8	108.8	108.8
Wheat gluten meal	100	100	77.8	77.8	77.8
Soybean meal	150	150	116.7	116.7	116.7
Soy protein concentrate	260	260	202	202	202
GemGel wheat starch[b]	100.8	69.9	245.5	194	212.2
Menhaden fish oil	195.2	195.2	195.2	195.2	195.2
Stay-C 35[c]	3	3	3	3	3
Choline Cl 50%	5	5	5	5	5
Vitamin premix[d]	15	15	15	15	15
TM salt[e]	1	1	1	1	1
Taurine	5	5	5	5	5
dl-Methionine	0	1.1	0	4.5	4.5
Lysine HCl	0	16.4	0	23.7	23.7
Threonine	0	0	0	5.1	5.1
Glycine	0	13.4	0	18.2	0
Dicalcium phosphate	25	25	25	25	25
Analyzed composition					
Crude protein (N*6.25) (g/kg)	460	490	369	415	409
Gross energy (MJ/kg)	23.7	23.8	23.2	23.5	23.2

[a] Diet designations: 45CP = 45% crude protein from intact sources; 45AA = 45% crude protein from intact sources plus supplemental Lys, Met, and Gly; 35CP = 35% crude protein from intact sources; 35AA = 35% crude protein from intact sources plus supplemental Lys, Met, Thr, and Gly; 35AA-Gly = 35% crude protein from intact sources plus supplemental Lys, Met, and Thr.
[b] Pregelatinized wheat starch, Manildra Group USA, Shawnee Mission, Kansas, USA.
[c] Vitamin C as Rovimix® Stay-C® 35, DSM Nutritional Products, Basel, Switzerland.
[d] Contributed per kg of diet: vitamin A (as retinol palmitate), 30,000 IU; vitamin D_3, 2160 IU; vitamin E (as DL-%-tocopheryl-acetate), 1590 IU; niacin, 990 mg; calcium pantothenate, 480 mg; riboflavin, 240 mg; thiamin mononitrate, 150 mg; pyridoxine hydrochloride, 135 mg; menadione sodium bisulfate, 75 mg; folacin, 39 mg; biotin, 3 mg; vitamin B_{12}, 90 μg.
[e] Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3.

define this balance. Gaylord and Rawles (2005) conducted a study that supported the use of the ideal protein concept in aquaculture feeds. A diet based on pet food grade poultry by-product meal was formulated on an ideal protein basis, and fed to hybrid striped bass for 10 weeks. Fish fed the amino acid supplemented, fish meal-free diet, had growth equivalent to bass fed a fish meal based diet.

The ability to improve fish growth and feed efficiencies through supplementation of one or more essential amino acids has been demonstrated previously to allow for reductions in total dietary protein. Viola et al. (1992) demonstrated that total dietary protein could be reduced when lysine was supplemented to carp diets, and Cheng et al. (2003b) demonstrated that dietary crude protein could be reduced from 42 to 37% in fish meal based diets for rainbow trout when lysine, methionine, threonine and tryptophan were supplemented. Other research to demonstrate the ability of lysine supplementation (Gaylord et al., 2002) or lysine and methionine supplementation (Li and Robinson, 1998) to reduce total dietary crude protein needs in channel catfish was ineffective. One reason for a lack of positive results, in some cases, may have been that another amino acid was first limiting instead of lysine or methionine.

Therefore, the current trial was performed to determine if supplementing diets with amino acids based on a theoretical ideal amino acid composition would improve production characteristics in rainbow trout fed a plant protein-based diet. In addition, a reduction of the total protein content of the plant protein-based diets without sacrificing commercially relevant performance characteristics was also evaluated.

2. Materials and methods

2.1. Animal husbandry, diet formulation and experimental design

Diets were formulated to contain 45% crude protein (45 series diets) and 35% crude protein (35 series diets) (Table 1) and to provide protein in the same ratios from each of the ingredients. Amino acids were calculated on an available amino acid basis (Gaylord unpublished results). Diets 45AA and 35AA were supplemented with methionine, lysine and glycine or methionine, lysine, glycine and threonine, respectively, increasing the crude protein levels to 49.0 and 41.5%, respectively, while the 35AA-Gly diet was supplemented with methionine, lysine and threonine only. Levels of supplementation were calculated to provide amino acid levels equivalent to 45% crude protein from rainbow trout muscle protein (Table 2), but analyzed levels fell short of calculated supplementation levels. All diets were manufactured using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 s exposure to an average of 127 °C in the sixth extruder barrel sections. The die plate was water cooled to an average temperature of 60 °C and pressure at the die head averaged 260 psi. The 3.0 mm pellets were then dried in a pulse-bed drier (Buhler AG, Uzwil, Switzerland) for 25 min at 102 °C with a 10 min cooling period. Final moisture levels were less than 7%. All oil was included in the mix rather than top-coated.

Twenty-five fish with a mean initial weight of approximately 20 g per fish (mean tank weight 499.4 g ± 8.1, mean ± S.D.) were stocked in each of 15, 140 L tanks receiving flow-through spring water at 15 C at the Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho. A domesticated strain of rainbow trout, *Oncorhynchus mykiss* (House Creek strain, College of Southern Idaho, Twin Falls, Idaho) were stocked, and the fish conditioned to the culture system for one week and fed a commercial trout feed containing 45% crude protein and 16% lipid. A fixed photoperiod, controlled by timers and fluorescent lights, was followed (14 h light:10 h dark). The experimental protocol was approved by the University of Idaho's Animal Care and Use Committee.

Each diet was randomly assigned to three tanks of fish. Fish were fed by hand to apparent satiation three times per day, six days per week. All fish within a tank were counted and weighed as a group every three weeks.

2.2. Sample collection

At the termination of the 12-week experiment, all fish were counted and weighed. Three fish per tank were bled, euthanized and dissected to determine body condition indices approximately 16 h postprandial. Blood was collected by caudal puncture using heparinized syringes and plasma was separated for amino acid analysis.

Table 2
Analyzed dietary amino acid concentration[a]

Amino acid	Diet					
	Trout muscle[b]	45CP	45AA	35CP	35AA	35AA-Gly
	g/kg dry diet					
Ala	25.9	23.1	22.8	18.2	18.1	18.3
Arg	35.0	24.9	25.4	20.1	19.5	20.9
Asx	42.2	44.8	45.4	36.8	36.4	36.5
Glx	63.7	109.6	113.7	88.1	90.0	92.1
Gly	22.7	16.3	27.7	12.6	29.8	12.6
His	10.2	9.5	9.5	7.2	6.7	9.7
Ile	20.8	20.5	21.0	16.4	15.8	17.1
Leu	36.6	43.5	43.9	34.6	34.0	34.4
Lys	39.1	17.5	26.3	11.5	21.7	30.9
Met	14.2	6.5	7.7	5.2	8.3	9.9
Phe	18.8	24.6	25.2	20.0	19.5	21.1
Tyr	16.0	15.8	16.9	13.2	12.9	13.9
Ser	18.6	21.1	20.9	17.0	17.0	17.3
Thr	21.5	16.0	16.2	12.7	16.1	18.6
Val	23.1	22.3	22.7	17.9	17.1	18.5

[a] Diet designations: 45CP = 45% crude protein from intact sources; 45AA = 45% crude protein from intact sources plus supplemental Lys, Met, and Gly; 35CP = 35% crude protein from intact sources; 35AA = 35% crude protein from intact sources plus supplemental Lys, Met, Thr, and Gly; 35AA-Gly = 35% crude protein from intact sources plus supplemental Lys, Met, and Thr.
[b] Amino acid composition of 450 g rainbow trout muscle protein.

The condition indices determined included:

Muscle ratio (MR) fillet mass with ribs (g)*100/fish mass (g)
Hepatosomatic index (HSI) liver mass (g)*100/fish mass (g)
Intraperitoneal fat ratio (IPF) peritoneal fat mass (g)*100/fish mass (g).

2.3. Sample analyses

Plasma and dietary amino acids were quantified according to Fleming et al. (1992) with an Agilent 1100 series HPLC. To determine dietary amino acids, diets were capped with nitrogen and hydrolyzed in 6 M HCl at 110°C for 16 h (AOAC, 1995). For plasma amino acids, plasma proteins were precipitated with 1.5 M perchloric acid, followed by centrifugation at 17,500 ×g for 5 min. All samples were derivatized with o-phthaldialdehyde (P0532, Sigma-Aldrich Co., St. Louis, MO) immediately prior to injection on a 5 µm Agilent Hypersil AA ODS column (part number 79916AA-572, Agilent Technologies, Palo Alto, CA) using an automated injection sequence.

Feed intake, weight gain, and feed conversion ratio (FCR) were calculated according to the following formulas:

Feed intake g dry feed consumed*100/100 g body mass/day
Weight gain (final weight (g)−initial weight (g))*100/initial weight (g)
FCR g dry feed fed/g wet weight gain.

For the determination of whole body protein, individual amino acid, and energy retention, 10 fish were sampled at the time of stocking and three additional fish per tank were sampled at the end of the feeding trial. Protein, amino acid, and energy retention efficiencies were calculated as follows:

Protein retention efficiency (PRE) protein gain (g)*100/protein fed (g)
Amino acid retention efficiency (AARE) amino acid gain (g)*100/amino acid fed (g)
Energy retention efficiency (ERE) energy gain (MJ)*100/energy fed (MJ).

Dry matter of ingredients and diets was determined according to standard methods (AOAC, 1995). Crude protein (N×6.25) was determined by the Dumas method (AOAC, 1995) on a Leco nitrogen analyzer (TruSpec N, LECO Corporation, St. Joseph, MI, USA). Gross energy was determined by adiabatic bomb calorimetry (Parr 6300, Parr Instrument Company Inc., Moline, IL, USA).

2.4. Statistics

Differences among response variables were determined by analysis of variance (ANOVA) and deemed significant at $P<0.05$ (SAS software program Proc GLM, Version 7, SAS Institute, Cary, NC, USA). Where significant effects were found, comparisons among treatment means were performed using Tukey's means separation (Tukey, 1953; Kramer, 1956).

3. Results (Note: a percentage is not a rate!)

Analyzed values for 17 dietary amino acids are reported in Table 2. Glu and Gln are reported as Glx and Asp and Asn are reported as Asx since the acid hydrolysis procedure deamidates Asn and Gln to Asp and Glu, respectively. Growth, reported as g gained per fish and as a percent increase, was significantly affected by supplementation of amino acids (Table 3). No difference in growth as g gained was noted between fish fed diets 45CP and 35CP, but supplementation of amino acids improved growth of fish fed either protein level. Trout fed 35AA-Gly had the highest overall growth and were equivalent to trout fed the 45AA and 35AA diets. Feed conversion ratio was significantly higher for fish fed the 35CP diet than the other diets.

Table 3
Effect of diet on growth, feed performance, nutrient retention, whole body composition, condition indices and amino acid retention[1]

	45CP	45AA	35CP	35AA	35AA-Gly	$P>F$[2]
g gain	220.2^{bc}	245.5^{a}	215.0^{c}	240.6^{ab}	247.7^{a}	0.0456
% Gain[3]	1108^{bc}	1222^{ab}	1068^{c}	1206^{ab}	1246^{a}	0.0368
FCR[4]	1.20^{a}	1.14^{a}	1.32^{b}	1.20^{a}	1.11^{a}	0.0134
FI[5]	2.41^{ab}	2.33^{b}	2.61^{a}	2.45^{ab}	2.28^{b}	0.0610
PRE[6]	28.1^{c}	31.2^{bc}	30.1^{bc}	34.2^{ab}	38.2^{a}	0.0001
ERE[7]	37.4	35.9	36.3	37.4	38.7	0.8084
WBCP[8]	15.3^{cd}	17.1^{ab}	14.4^{d}	16.2^{bc}	17.3^{a}	0.0001
WBE[9]	2400	2230	2475	2324	2316	0.2677
HSI[10]	1.54^{b}	1.28^{c}	1.83^{a}	1.28^{c}	1.23^{c}	0.0001
IPF[11]	3.72^{ab}	2.82^{c}	3.97^{a}	2.80^{c}	3.10^{bc}	0.0023
MR[12]	45.2^{b}	49.9^{a}	43.1^{b}	48.8^{a}	49.4^{a}	0.0001
Amino acid retention efficiencies (%)[13]						
Lys	49.1^{b}	35.6^{c}	53.4^{a}	30.7^{d}	35.6^{c}	0.0001
Met	48.2	52.1	52.0	43.8	47.1	0.2528
Thr	34.9	38.8	36.0	34.7	37.7	0.0892
Gly	45.2^{b}	27.4^{c}	46.9^{b}	26.0^{c}	59.8^{a}	0.0001
Ile	24.4^{c}	29.3^{b}	26.3^{c}	34.7^{a}	35.5^{a}	0.0001
Leu	19.0^{c}	22.1^{b}	20.4^{bc}	26.1^{a}	27.5^{a}	0.0001

[1]Initial tank weights were 499.4 g±8.1 g with no significant differences between tanks, for all analyses $n=3$.
Diet designations: 45CP = 45% crude protein from intact sources; 45AA = 45% crude protein from intact sources plus supplemental Lys, Met, and Gly; 35CP = 35% crude protein from intact sources; 35AA = 35% crude protein from intact sources plus supplemental Lys, Met, Thr, and Gly; 35AA-Gly = 35% crude protein from intact sources plus supplemental Lys, Met, and Thr.
[2]Probability associated with the F statistic for the factorial ANOVA. Values not sharing a common superscript within rows are significantly different from one another at $P<0.05$.
[3]% Gain=(final weight (g)−initial weight (g))*100/initial weight (g).
[4]FCR: feed conversion ratio=g dry feed fed/g wet weight gain.
[5]FI: feed intake=g feed consumed*100/100 g body mass/day.
[6]PRE: protein retention efficiency=g protein gain*100/g protein fed.
[7]ERE: energy retention efficiency=kcal energy gain*100/kcal energy fed.
[8]WBCP, whole body crude protein=analyzed nitrogen*6.25.
[9]WBE, whole body gross energy.
[10]Hepatosomatic Index=liver mass (g)*100/fish mass (g).
[11]Intraperitoneal fat ratio=peritoneal fat mass (g)*100/fish mass (g).
[12]Muscle ratio=fillet mass (g)*100/fish mass (g).
[13]Amino acid retention efficiency=individual amino acid gain (g)*100/individual amino acid fed (g).

Protein retention efficiency was highest for fish fed the 35AA-Gly diet and lowest for fish fed the 45CP diet. Energy retention efficiency was not significantly altered by diet. Whole body crude protein was highest for fish fed the 35AA-Gly diet and lowest for fish fed the 35CP diet with fish fed other diets being intermediate. Whole body energy concentrations were not significantly affected by diet.

Relative liver size expressed as hepatosomatic index (HSI) was between 1.23 and 1.28% for fish fed the amino acid supplemented diets and were significantly lower than fish fed the 45CP and 35CP diets. Intraperitoneal fat ratio (IPF) was reduced in fish fed the amino acid supplemented diets compared to fish fed the 35CP diet. Muscle ratios were increased by amino acid supplementation at both protein levels compared to fish fed either the 35CP or 45CP diets.

Amino acid retention efficiencies for lysine decreased with lysine supplementation compared to the 45CP and 35CP diets (Table 3). Methionine retention efficiencies were unaffected by methionine supplementation to either the 35CP or 45CP diets. Glycine retention efficiencies were reduced when glycine was supplemented to either diet series; however, glycine retention efficiencies were significantly higher for fish consuming the 35AA-Gly diet compared to all other treatments. Amino acid supplementation also improved retention efficiencies of isoleucine and leucine relative to both the 35CP and 45CP diets.

Supplementation of lysine increased plasma lysine levels in the 45CP series and the 35CP series treatments (Table 4). Methionine supplementation only increased plasma methionine levels in the 35CP series treatments. Threonine supplementation had no effect on

Table 4
Effect of diet on plasma amino acid concentrations[1]

Plasma amino acids	45CP	45AA	35CP	35AA	35AA-Gly	$P>F$[2]
	nmol/mL					
Lys	120[b]	244[a]	100[b]	278[a]	269[a]	0.0092
Met	134[b]	158[b]	118[b]	301[a]	262[a]	0.0041
Thr	186	140	132	176	167	0.4333
Gly	508[bc]	644[b]	421[c]	1032[a]	337[c]	0.0002

[1]Diet designations: 45CP = 45% crude protein from intact sources; 45AA = 45% crude protein from intact sources plus supplemental Lys, Met, and Gly; 35CP = 35% crude protein from intact sources; 35AA = 35% crude protein from intact sources plus supplemental Lys, Met, Thr, and Gly; 35AA-Gly = 35% crude protein from intact sources plus supplemental Lys, Met, and Thr.
[2]Probability associated with the F statistic for the factorial ANOVA. Values not sharing a common superscript within rows are significantly different from one another at $P<0.05$.

plasma threonine levels, and glycine supplementation increased plasma glycine in the 35AA treatment over that of the unsupplemented treatments.

4. Discussion

The trend in trout feeds in the last 20 years has been toward maximizing growth through increased protein and energy concentrations. Protein content is a major driver in feed cost, however, a shift in both protein source and level is anticipated in response to changing ingredient markets. The ability to reduce dietary protein may be beneficial in reducing cost of production if growth and feed efficiencies can be maintained.

The amino acids lysine, methionine and threonine were chosen for supplementation in the current feeding trial because they were the most limiting in the formulations used, as well as, their commercial availability for potential economical supplements in aquafeeds. A discrepancy occurred between analyzed values and formulated values for the amino acids, but lysine and methionine were still the first and second limiting amino acids at 70.6 and 63.4% below the trout muscle values, respectively, in the 35% crude protein unsupplemented diet. Arginine may have become third limiting at 42.6% below trout muscle levels with threonine at forth limiting being 40.9% below trout muscle. Glycine was chosen as a potential supplement to the plant-based 35% crude protein diet due to its very low content in the plant-derived ingredients used and the positive effects that have been observed in poultry feeds (Dean et al., 2006).

Interestingly, in the current trial, trout fed the 45CP and 35CP diets had equivalent growth, protein retention efficiencies as well as plasma lysine, methionine, threonine and glycine levels. This was unexpected as the protein requirement of trout has been established to be between 42 and 48% from practical ingredients (Hardy, 2002). One might suspect that the fish were consuming equivalent total quantities of protein; however, feed consumption for the 35CP treatment was 2.61% compared to 2.41% for the 45CP treatment, which was not statistically different. Although this would only account for 0.91 g protein consumed per 100 g fish fed the 35CP diet versus 1.08 g protein consumed per 100 g fish fed the 45CP diet, equivalent amino acid intake may be suggested by equivalent levels of plasma lysine, methionine, threonine and glycine, as plasma amino acid levels have been shown to correlate with diet (Walton and Wilson, 1986; Schuhmacher et al., 1997).

Amino acid supplementation did improve growth performance in both the high and low protein diets, but greater improvements were observed with the low protein diets. Growth (g gain) was improved by 11.5% in fish fed the 45AA diets compared to those fed the 45CP diet, while growth increased by 12 and 15% for fish fed the 35AA and 35AA-Gly diets compared to the 35CP diet. These improvements are particularly relevant given the reduction in relative feed intake in the 35AA-Gly treatment compared to the 35CP treatment and demonstrate the ability of amino acid supplementation to improve nutrient retention. Rodehutscord et al. (2000) observed similar results in rainbow trout fed 35 and 55% CP diets in which wheat gluten and supplemental amino acids were the sole sources of protein. In that experiment, growth did not differ when fish were fed diets containing two different levels of protein, but equivalent levels of total lysine. Moreover, growth improved with increasing levels of dietary lysine regardless of total protein concentration. Rodehutscord et al. (2000) also noted that lysine retention efficiency increased more with the high protein diets than with the low protein diets as dietary lysine level increased. In the current study, reducing intact protein from 45 to 35% improved lysine retention from 49 to 53% but supplementing lysine reduced retention to 31 and 36%. The differences in lysine retention may be due to absolute differences in dietary lysine concentrations which ranged from 1.2 to 3.1% in the current trial and 0.96 to 1.8% in Rodehutscord et al. (2000). Therefore, supplemental levels in the current study may have exceeded the dietary requirement for maximum utilization efficiency. Interestingly, neither supplemental methionine nor threonine had any effect on their respective retention efficiencies. When isoleucine and leucine retention efficiencies were calculated, however, supplementation of lysine, methionine and threonine improved retention of those branched chain amino acids by 16–20% in the high protein diets and 35% in the low protein diets. One potential explanation for the differences in retention efficiencies may be that methionine and threonine were not over supplemented whereas lysine was over supplemented. As amino acid balance is refined, it may be possible to improve retention efficiencies as we observed with isoleucine and leucine; both exhibited improved retention efficiencies when test diets were balanced on an ideal protein basis.

Optimization of amino acid balance in aquafeeds is essential if optimal fish growth and production efficiencies are to be realized, particularly when feeding non-fish meal based diets (Gatlin et al., 2007). Formulations may be utilizing excess dietary protein to meet the amino acid requirements, and opportunities may exist to reduce total protein in fish feeds by supplementing a few economically feasible limiting amino acids. Viola and Lahav (1991) demonstrated that total dietary protein may be reduced from 30 to 25% when lysine was supplemented to carp diets. Cheng et al. (2003b) demonstrated that trout grew equally well when fed 37% as opposed to 42% crude protein when fish meal was reduced by 50% and lysine, methionine, threonine and tryptophan were supplemented to be equivalent to the 42% crude protein control diet. Yamamoto et al. (2005) showed that supplementation of all essential amino acids to a low-protein (35%) diet improved protein retention efficiency in trout from 35% in a 45% crude protein diet to 50% in a 35% crude protein diet. On the other hand, supplementing lysine to a predominately soybean meal channel catfish feed did not improve catfish growth (Gaylord et al., 2002). Similarly, Li and Robinson (1998) were unable to reduce total dietary crude protein from 32 to 24% with lysine supplementation or from 28 to 24% with lysine and methionine supplementation in channel catfish. One possible reason for the lack of improved growth with amino acid supplementation in catfish diets may have been that other amino acids became first limiting instead of lysine and/or methionine, even though the established amino acid requirements for catfish appeared to have been met (Li and Robinson, 1998).

Results of the current trial suggest that protein level in trout diets could be reduced from 46 to 40.9% crude protein (analyzed values) by supplementing with methionine, lysine and threonine on and ideal protein basis. This is a net reduction of 5.1 g of crude protein per 100 g diet based on analyzed nitrogen and is similar to that reported by Botaro et al. (2007) for Nile tilapia. When lysine, methionine and threonine were supplemented to Nile tilapia diets, digestible protein could be reduced from 27 to 24% without a reduction in growth. Viola et al. (1992) also observed no reduction in carp growth when dietary

crude protein was reduced from 30 to 25% and lysine plus methionine were supplemented on an ideal protein basis.

It has been widely acknowledged that feeding diets with amino acid deficiencies results in altered protein deposition and excess energy deposition as fat in the liver, fillet or peritoneal cavity. Cheng et al. (2003a) observed increased whole-body crude protein levels in rainbow trout with increased lysine supplementation up to 1.8% of the diet, while Cheng et al. (2003b) observed increased whole-body protein concentrations with decreased fat deposition when lysine was supplemented to a reduced fish meal diet for rainbow trout. Cheng et al. (2003b) also noted reductions in total ammonia nitrogen excretion as lysine supplementation increased in the low protein diets. Although they did not report protein retention efficiencies, decreasing ammonia levels suggest increased nitrogen retention and corroborate the results of the current trial in which amino acid supplementation improved total nitrogen retention from 30% in the 35AA to 38% in the 35AA-Gly treatment. Similar improvements were observed by Cheng et al. (2003a), who reported that protein retention efficiency improve from 25 to 33% when diets containing 43% crude protein, 15% fish meal, and 1.5% lysine were supplemented with lysine to obtain a total level of 2.3% lysine in the diet.

In the current study, amino acid balance and protein level of the diets did not affect energy retention of rainbow trout, whereas, storage depots of fat was affected. Less fat was deposited in the muscle when amino acids were not supplemented, as demonstrated by the lower muscle ratio in fish fed the 45CP and 35CP diets relative to all the amino acid supplemented treatments. To some extent, the excess energy consumed by these fish that could not be deposited as muscle protein was, presumably, deposited as fat in the peritoneal cavity or liver.

Although the diets were not formulated to be isocaloric on a digestible energy basis, protein to energy ratio did change in the formulations. The combined effects of changing protein and energy ratios and simultaneously balancing the amino acid profile are difficult to decipher. Encarnacao et al. (2004) determined that supplementing deficient trout diets with lysine would improve protein retention efficiency and growth, and altering the digestible energy content of the diets from 16 to 20 MJ/kg only appeared to affect protein retention efficiency when lysine was sufficient. There also was no affect of altered digestible energy concentration of the diet on the lysine requirement estimate in that study. It did appear that protein retention efficiency would be improved through both improving dietary amino acid balance as well as potential protein sparing with high energy diets. Further research to address optimized protein to energy balance with diets balanced for amino acid profiles are needed to ensure maximized efficiency.

The profitability of trout production will partially hinge on the ability to formulate economically viable feeds that support efficient growth and healthy fish. Refinements in requirement estimates will allow formulation of trout feeds with increased precision, minimal nutrient waste, and lower cost because over formulation to meet limiting amino acid requirements will be reduced or eliminated. The current trial demonstrates that balancing dietary amino acids in plant-based feeds for rainbow trout can reduce nitrogen losses from the fish and improve feed conversion ratios while maintaining rapid growth. However, further research is necessary to refine available amino acid balance that maximize protein gain and amino acid retention, and address the economic value of alternative protein sources.

Acknowledgements

We wish to thank ARS technicians April M. Teague and Lorrie Van Tassel, ARS SCEP student, G. Scott Snyder, and University of Idaho personnel Mike Casten and Lucas Porter for their assistance with this study. This study was funded by the USDA/Agricultural Research Service Project Numbers 5366-21310-002-00D.

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture (USDA).

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